10/636,079

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 39/21 — A1

(11) International Publication Number: WO 92/00098

(43) International Publication Date: 9 January 1992 (09.01.92)

(21) International Application Number: PCT/EP91/01225

(22) International Filing Date: 1 July 1991 (01.07.91)

(30) Priority data:
545,064 29 June 1990 (29.06.90) US

(71)(72) Applicant and Inventor: ZAGURY, Daniel [FR/FR]; 22, avenue Mozart, F-75016 Paris (FR).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: IMBERT, Jean-Claude [FR/FR]; 91 bis, rue du Cherche-Midi, F-75007 Paris (FR). SALAUN, Jean-Jacques [FR/FR]; Parc Berger-Cybèle, 105, bd du Cabot, F-13009 Marseille (FR). ZIRIMWAMBA, Lurhuma [ZR/ZR]; Campus Universitaire du Mont Amba, Kinshasa II (ZR).

(74) Agent: CABINET GERMAIN ET MAUREAU; BP 3011, F-69392 Lyon Cédex 03 (FR).

(81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CI (OAPI patent), CM (OAPI patent), DE, DE (European patent), DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GN (OAPI patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL, NL (European patent), NO, PL, RO, SD, SE, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent), US.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHODS OF INDUCING IMMUNE RESPONSE TO AIDS VIRUS

(57) Abstract

A safe, effective vaccine will raise antibodies against several parts of the genome of the HIV virus. A protocol for the administration of the vaccine will cause the production of an immune response to protect an individual against several strains of HIV virus. The immunotherapy protocol will cause the individual to develop protective immunity against HIV blocking viral expansion and dissemination in infected individuals. New improved immunogenic compositions are also provided.

Cito - Thus

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**ES**	Spain	**MG**	Madagascar
AU	Australia	**FI**	Finland	**ML**	Mali
BB	Barbados	**FR**	France	**MN**	Mongolia
BE	Belgium	**GA**	Gabon	**MR**	Mauritania
BF	Burkina Faso	**GB**	United Kingdom	**MW**	Malawi
BG	Bulgaria	**GN**	Guinea	**NL**	Netherlands
BJ	Benin	**GR**	Greece	**NO**	Norway
BR	Brazil	**HU**	Hungary	**PL**	Poland
CA	Canada	**IT**	Italy	**RO**	Romania
CF	Central African Republic	**JP**	Japan	**SD**	Sudan
CG	Congo	**KP**	Democratic People's Republic of Korea	**SE**	Sweden
CH	Switzerland			**SN**	Senegal
CI	Côte d'Ivoire	**KR**	Republic of Korea	**SU**	Soviet Union
CM	Cameroon	**LI**	Liechtenstein	**TD**	Chad
CS	Czechoslovakia	**LK**	Sri Lanka	**TG**	Togo
DE	Germany	**LU**	Luxembourg	**US**	United States of America
DK	Denmark	**MC**	Monaco		

METHODS OF INDUCING IMMUNE RESPONSE TO AIDS VIRUS

SUMMARY OF THE INVENTION

This invention provides means of inducing an immune response to viral antigens as a means to protect against infection. The methods of the invention also induce immune response against viral antigens in infected individuals, thereby slowing the progress of the disease. Of particular importance is protection against human immuno-deficiency virus (HIV), the causative agent of acquired immune deficiency syndrome (AIDS). However, while immunization against the HIV retrovirus is exemplified, such exemplification should not be considered as a limitation on the invention.

BACKGROUND OF THE INVENTION

It is known that immune activation of HIV infected T_4 lymphocytes is required for viral release. These cells act as the source for expansion and dissemination of virions in the organisms leading to AIDS. (Zagury, et al., Science, 231, 850-853 (1986)) The viral release which leads to death of infected cells is preceded by a stage where HIV signals are present in the cell membrane. This immunogenic stage is important as a trigger for the cellular reaction against HIV. The reaction leads to the destruction of the infected cells. (Zagury, et al., Proc. Nat. Acad Sci. USA, Vol. 85, pp. 3570-3574 (1988).

Previous immunization protocols have been aimed at establishing a candidate vaccine (rV) expressing HIV proteins followed by a booster of autologous cells infected with rV which have subsequently been fixed. These lead to both humoral and cellular immune responses to HIV. (Zagury, et al., Nature 332, p. 723 (1988).)

The development of a safe and effective vaccine against the AIDS (HIV) virus has become a high priority concern of the scientific and medical community. Rapid progress in the isolation, cloning, and sequencing of the entire genome has shown the remarkable propensity of the HIV strains to mutate, particularly within the viral envelope gene. Since viral envelope proteins are often

the target for neutralizing antibodies, this extensive variation may play an important role in the interaction between the virus and the host's immune system. For some viruses, such as influenza, rapid mutation is an important means of escape from neutralizing antibodies. Such mutation results in successive waves of influenza epidemics among previously infected populations. For visna virus, a sheep retrovirus, the mutation rate is believed to be so rapid as to allow antibody escape during the course of a single chronic infection. If similar mutants arise in humans infected with viral infections, HIV being one example, during the course of multiple rounds of infection, it would be difficult to imagine a vaccine antigen that could keep pace with all of the possible variants. Under such circumstances it would be impossible to develop an effective vaccine.

In spite of the observed rapid mutation rate of any virus, it is possible that a virus cannot mutate at certain sites, particularly those serving essential viral functions. For example, the CD4 binding site of HIV has been mapped to three relatively conserved regions of gp120. Divergent isolates bind soluble CD4 and are inactivated by it, suggesting conservation of the CD4 binding site. Presumably, if neutralizing antibodies were directed against this site or another site responsible for a critical viral function, such antibodies would be active against numerous clinical isolates of the virus. A vaccine capable of eliciting antibodies against a broad spectrum of HIV strains is needed.

Previous work using retroviral immunogenic signals in their native configuration linked to exogenous carriers and/or in water-soluble adjuvants such as alum is well known. These immunizing preparations did not, however, trigger a significant anti-viral group-specific reaction. The lack of group specific response is particularly important when the infectious agent mutates readily and/or when the species is characterized as composed of a large number of strains showing differing antigenic properties.

DESCRIPTION OF THE INVENTION

It is the purpose of the present invention to provide a safe, effective vaccine that will raise antibodies against several parts of the genome of the HIV virus.

It is a further purpose of the invention to provide protocol for administration of a vaccine that causes the production of an immune response which will protect the individual against several strains of HIV virus.

It is also a purpose of the invention to provide an immunotherapy protocol that will cause the patient to develop protective immunity against HIV, which will block viral expansion and dissemination in infected individuals.

It is, additionally, a purpose of the invention to provide new, improved immunogenic compositions.

It has been possible to obtain antibodies against HIV by infecting autologous cells (patient cells) with recombinant virus (rV) carrying HIV segments (envelope, gag, or pol). An example of such a recombinant virus is the virus of Moss. Mackett, et al., J. Virol. 49, 857-864.) Another method of developing immune response to HIV may be accomplished by incubating autologous cells with synthetic peptides to allow peptides to form complexes with HLA antigens on the cell surface, then fixing the cells by usual methods known in the art, e.g., fixation with paraformaldehyde or glutaraldehyde (Zagury, et al, Nature, 332, pp. 728-731 (1988)).

It is also possible to raise an immune response by the administration of compositions containing only the cell-free membranes from autologous cells that have been incubated with the appropriate peptides or have been infected with virus containing recombinant HIV nucleic acid sequences. (See Zagury, Nature, above.) The sequences to which the autologous cells are exposed are usually from the envelope, gag, or pol proteins.

More recently, an immune response has been obtained by the administration of compositions containing

"cocktails" of several free peptides representing sequences found in HIV proteins. These peptides in aqueous solution can be added to oils to form an emulsion. These compositions have immunogenic properties and can be administered to individuals either in conjunction with administration of preparations containing treated autologous cells or cell free membranes. However, the peptide-containing emulsions can also be administered alone as a means of raising an immune response in the individual The emulsions are administered parenterally, intramuscularly or subcutaneously.

There are several advantages in using the compositions of the present invention either alone or in conjunction with a protocol that includes use of autologous cells that have been exposed in vitro to HIV antigens, or cell membranes obtained therefrom. 1) The "peptide cocktail" approach allows the use of selected amino acid sequences. These sequences may be chosen from regions of the protein that are conserved across the various strains of the organism. Additionally, peptide sequences from several strains may be chosen so that a broad range of viral strains may be used to give broad group protection against HIV virus.

The peptides may be prepared by any means known in the art, such as by recombinant means or by the Merrifield process. The process of preparing the peptides by synthetic means provides not only reliability, but also provides certain economic advantages.

Peptides should be of about 8 to 40 amino acids, with peptides of 12 to 30 amino acids preferred. In addition to the emulsion containing the peptides, protein fragments having molecular weights of over 10,000 can be added to the emulsion or can be given separately from the peptides to stimulate increased antibody response. Of course, adjuvants and other additives known in the art may also be added to the peptide-containing emulsions. Some of the larger segments that can be used include gp160,

gp41, gp^{366}, gp24, reverse transcriptase (RT), Tat protein, and protease.

While peptides from several strains and differing locations in the HIV proteins can be used, particularly preferred sites are listed below:

Y-N-K-R-K-K-I-H-I-G-P-G-R-A-F-Y-T-T-K-N-I-I-G

R-I-G-P-G-R-A-F-V-T-I-G-K

Q-K-V-G-K-A-M-Y-A-P-P-I-S-G

D-M-V-E-Q-M-H-E-D-I-I-S-L-W-D-Q-S-L-K-P-C

W-G-I-K-Q-L-Q-A-R-I-L-A-V-E-R-Y-L-K-D-Q

C-K-I-K-Q-I-V-K-M-W-Q-C-V-G-Q-A-I-Y

N-T-R-K-S-I-R-I-Q-R-G-P-G-R-A-F-V-T-I-G-K-I-G

N-N-T-R-K-S-I-T-K-G-P-G-R-V-I-Y-A-T-G-Q-I-I-G

N-N-V-R-R-S-L-S-I-G-P-G-R-A-F-R-T-R-G-K-I-I-G

R-I-G-P-G-R-A

G-P-G-R-A-F-V-T-I-G-K

N-Y-T-R-K-S-V-R-I-G-P-G-Q-A-F-Y-A-T-G-D-I-I-G

Q-N-T-R-Q-R-T-P-I-G-L-G-Q-S-L-Y-T-T-R-S-R-I-S

N-N-T-R-R-G-I-H-F-G-P-F-Q-A-L-Y-T-T-G-I-I-V-G

EXAMPLES

Example 1

AIDS or ARC (aids-related complex) patients with 150 - 400 T4 cells per mm^3 were immunized with fixed autologous B cells transformed with EBV (Epstein-Barr Virus) and infected with recombinant vaccinia expressing HIV proteins (env, gag, and pol) on the surface of infected cells. The fixed cells were given by intravenous slow drip infusion (5-7 x 10^7 cells), intramuscularly (10 x 10^7 cells in water suspension in animal oil (squalene, Montanide 708) or subcutaneously.

The vaccinia vector used is the non-neurotropic strain Lister and the recombinant is produced by the method of Moss (see Mackett, supra.) The patients underwent biweekly physical examination. Blood samples were drawn for preparation of serum and cells for biological investigations including virus neutralizing antibody, T4 cell

count, cell mediated immunity, and cell mediated cytotoxicity.

Example 2

AIDS or ARC patients with 150 - 400 T4 cells per mm^3 received the preparation described in example 1 in conjunction with AZT (600 mg per day). Other conditions were similar to those described in example 1.

Example 3

AIDS or ARC patients with 150 - 400 T4 cells per mm^3 received treatment as described in example 1 along with discontinuous AZT at low doses (600 mg per day for 30 days every 90 days). Other conditions were similar to those described in example 1.

Example 4

Asymptomatic HIV infected individuals with >500 T4 cells per mm^3 received the treatment described in example 1. Other conditions were similar to those described in example 1.

Example 5

AIDS or ARC patients with 150 - 400 T4 cells per mm^3 received a mixture of synthetic HIV peptides comprising peptides which constituted immunodominant sites of env, gag, pol (peptide 342-350 according to Ratner). Immunization protocols and patient follow up were as described in example 1.

Example 6

Patients described in example 5 were given a continuous low dose of AZT (600 mg/day). Other conditions were similar to those described in example 5.

Example 7

Patients treated as described in example 5 were given a discontinuous low dose of AZT (600 mg/day for 30 days every 90 days). Other conditions were similar to those described in example 5.

Example 8

Asymptomatic HIV infected individuals with >500 T4 cells per mm^3 were treated as described in example 5.

Example 9

Seronegative Patients were treated as described in example 5 except that the synthetic peptides administered were protectively encapsulated as a water-in-oil emulsion. All other conditions were as described in example 5.

Example 10

Seronegative Patients were given synthetic peptides as in example 5 except that the peptides were administered as free peptides.

Example 11

Seronegative Patients were given synthetic peptides representing HIV epitopes wherein the peptides were covalently linked to an immuno-enhancing moiety. All other conditions were as described in example 5.

Example 12

Peptide derivatives having hydrophobic groups consisting of tripalmitoyl cysteine, dipalmitoyl lysine, or a non-viral peptide of alpha helix configuration were administered in accord with the protocol used to administer the peptides in example 5.

Example 13

Peptides were administered in accord with example 5. However, the patients were also given recombinant vectors containing HIV nucleic acid sequences at separate sites in conjunction with the peptides.

Example 14

Peptides were given in a composition containing recombinant live vectors containing HIV nucleic acid sequences mixed with the synthetic peptides which represented HIV immunodominant epitopes protectively encapsulated as a water in oil emulsion. The composition was given in the manner described in example 5.

Materials: Montanide is a product of SEPPIC, a division of Cosmetique-Pharmacie, 70, Champs-Elysees, 75008 Paris, France, and is obtainable therefrom.

WHAT IS CLAIMED IS:

1. A composition of matter comprising peptides of 8-40 amino acids representing HIV epitopes protectively encapsulated as a water in oil emulsion.

2. The composition of claim 1, wherein the peptides are free peptides.

3. The composition of claim 1, wherein the peptides are covalently linked to an immunologically enhancing moiety.

4. The composition of claim 3, wherein the enhancing moiety is a hydrophobic segment.

5. The composition of claim 4, wherein the hydrophobic segment is selected from the group consisting of tripalmitoyl cysteine, dipalmitoyl lysine, and a non-viral peptide of alpha helix configuration.

6. The composition of claim 1, further comprising a surfactant for stabilizing the emulsion.

7. The composition of claim 1, further comprising HIV immunogenic protein or protein fragments of molecular weight over 10,000.

8. The composition of claim 1, further comprising recombinant live vectors which express recombinant proteins or epitopes of HIV.

9. A composition of matter comprising autologous EBV (Epstein-Barr Virus) transformed B-cells infected with recombinant virus expressing HIV epitopes which have been fixed.

10. A composition of matter comprising autologous EBV (Epstein-Barr Virus) transformed B-cells carrying HIV epitopes at the cell surface which have been fixed.

11. A method of inducing immune response to HIV by administration of an immunogenic effective amount of the composition of claim 1.

12. The method of claim 11, wherein a recombinant live vector containing HIV nucleic acid sequences is administered mixed with a peptide-containing emulsion of the composition of claim 1.

13. The method of claim 11, wherein a recombinant

vector containing HIV nucleic acid sequence is administered along with at least one peptide at a separate site.

14. The method of claim 11, used as a part of immunotherapy in AIDS and ARC patients.

15. The method of claim 12, used as part of a vaccine protocol for immunoprophylaxis in HIV infected asymptomatic individuals.

16. A method of inducing an immune response by administration of an effective amount of a composition of claim 9 or 10 as a part of immunoprophylaxis or immunotherapy for AIDS and ARC patients.

17. The method of claim 16, wherein there is administered additionally a composition of claim 1.

18. The method of claim 11 or 12, which is used as part of a vaccine protocol.

19. The composition of claim 1, further comprising a surfactant to stabilize the emulsion.

20. The composition of claim 1 or 2, further comprising protein or protein fragments of molecular weight over 10,000, such as Env gp AGO.

21. The composition of claim 1, further comprising recombinant virus which expresses recombinant proteins of HIV.

22. The method of claim 11, wherein the peptides are administered in oil subcutaneously or intramuscularly.

23. A composition comprising free peptides of 8 to 40 amino acids representing HIV epitopes, protectively encapsulated as a water-in-oil emulsion for administration to induce an immune response to HIV.

24. The composition according to claim 23, which further comprises a recombinant live vector containing HIV nucleic acid sequences.

25. The composition according to claim 23, wherein a recombinant vector containing HIV nucleic acid sequences and at least one peptide is administered separately from said free peptides.

26. The composition according to claim 23, for use as part of immunotherapy in AIDS and ARC (Aids Related

Complex) patients.

27. The composition according to claim 24, for use as part of the vaccine protocol for immunoprophylaxis in HIV infected asymptomatic individuals.

28. A composition comprising autologous EBV (Epstein-Barr Virus) transformed B cells infected with recombinant virus expressing HIV epitopes which have been fixed or EBV transformed B cells carrying HIV epitopes at the cell surface which have been fixed, for use in inducing an immune response as part of immunoprophylaxis or immunotherapy for AIDS and ARC patients.

29. The composition of claim 28, for use with the composition of claim 23 to induce an immune response as part of immunoprophylaxis or immunotherapy for AIDS and ARC patients.

30. The composition of claims 23 and 24, for use as part of a vaccine protocol.

31. Use of a composition comprising free peptides of 8 to 40 amino acids representing HIV epitopes, protectively encapsulated as a water-in-oil emulsion, in the manufacture of a medicament for inducing an immune response to HIV.

32. The use according to claim 31, wherein the composition further comprises a recombinant live vector containing HIV nucleic acid sequences.

33. Use of a composition comprising free peptides of 8 to 40 amino acids representing HIV epitopes, protectively encapsulated as a water-in-oil emulsion in the manufacture of a medicament for use as part of immunotherapy in AIDS and ARC patients.

34. Use of a composition comprising free peptides of 8 to 40 amino acids representing HIV epitopes protectively encapsulated as a water-in-oil emulsion and a recombinant live vector containing HIV nucleic acid sequences in the manufacture of a medicament as part of a vaccine protocol for immunoprophylaxis in HIV infected asymptomatic individuals.

35. Use of a composition comprising autologous

EBV (Epstein-Barr Virus) transformed B cells infected with recombinant virus expressing HIV epitopes which have been fixed or EBV transformed B cells carrying HIV epitopes at the cell surface which have been fixed, in the manufacture of a medicament for inducing an immune response as part of immunoprophylaxis or immunotherapy for AIDS and ARC patients.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 91/01225

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 A 61 K 39/21

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	C 12 N A 61 K C 07 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	EP,A,0346022 (MEDICAL RESEARCH COUNCIL) 13 December 1989, see page 5, lines 1-12; page 2, lines 43-51	9
Y		1-8
P,X	Chemical Abstracts, volume 114, no. 3, 21 January 1991 (Columbus, Ohio, US), M. Loleit et al.: "Conjugates of synthetic lymphocyte-activating lipopeptides with segments from HIV proteins induce protein-specific antibody formation", see page 527, abstract 22025m, & Biol. Chem. Hoppe-Seyler 1990, 371(10), 967-75	1-7,20
P,Y		8,22-34

-/-

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
31-10-1991	13. 12. 91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. PEIS M. Peis

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	Nature, volume 332, 21 April 1988, D. Zagury et al.: "A group specific anamnestic immune reaction against HIV-1 induced by a candidate vaccine against AIDS", pages 728-731, see page 728, right-hand column	9,10,28 -29,35
Y		1-8,30-34
Y	US,A,4384995 (STEVENS) 24 May 1983, see column 29, lines 35-48; column 55, line 37 - column 56, line 51	1-8,22-34
Y	EP,A,0339504 (DUPONT DE NEMOURS) 2 November 1989, see page 3, line 55 - page 4, line 1	1-7
Y	Advances in Veterinary Science and Comparative Medicine, volume 33, Academic Press, Inc., A. Altman et al.: "Immunomodifiers in vaccines", pages 301-343	1-8,22-34
Y	Nature, volume 326, 19 March 1987, D. Zagury et al.: "Immunization aganst AIDS in humans", pages 249-250, see page 249, third column, last paragraph	8

FURTHER INFORMATI CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 11-18,22 are directed to a method of treatment of the human body, the search has been carried out also for these claims and based on the alleged effects of the composition.

2. [ ] Claim numbers because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. [ ] Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this International application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. EP 9101225 SA 48771

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 04/12/91
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A- 0346022	13-12-89	GB-A-	2220939	24-01-90
		JP-A-	2036198	06-02-90
US-A- 4384995	24-05-83	US-A-	4302386	24-11-81
		CA-A-	1223206	23-06-87
		US-A-	4526716	02-07-85
		US-A-	4762913	09-08-88
EP-A- 0339504	02-11-89	AU-A-	3332089	02-11-89
		JP-A-	2160800	20-06-90